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Effects of Dawa-ul-Kurkum, a Unanipolyherbal preparation, in anti-tubercular drug induced hepatotoxicity in rats and its possible mechanisms

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Abstract
Anti-tubercular drug therapy is associated with hepatotoxicity which sometimes leads to hospitalization and life-threatening problems. The effects of a polyherbal Unani formulation, Dawa-Ul-Kurkum were evaluated on anti-tubercular (TB) drugs induced hepatotoxicity in rats and its possible mechanisms were investigated. Liver damage was induced in Wistar rats by daily oral administration of a combination of anti-TB drugs for 28 days and the effects of various drug treatments were assessed on morphological, biochemical and histological markers of liver toxicity. In the vehicle treated experimental group, anti-TB drugs induced significant derangements in liver function parameters and histopathological examination showed hepatocyte degeneration, focal necrosis, perivascular infiltration of inflammatory cells and mild vasodilation. Pretreatment with Dawa-Ul-Kurkum (DK) showed marked protective effects against the Anti-TB drugs induced biochemical and histopathological derangements of liver function. Similar effects were also seen after the hydroalcoholic extract of DK (HA), though to a lesser extent. The hepatoprotective effects of DK and HA were comparable to that seen after silymarin therapy. Liver damage induced by Anti-TB drugs was associated with elevated levels of MDA and NOx whereas; GSH levels were reduced, as compared to controls. Pretreatments with DK and HA induced differential degrees of attenuations in these oxidative stress parameters. The results validate the hepatopotective effects of Dawa-Ul-Kurkum in Anti-TB drugs induced hepatotoxicity and suggest that attenuation of oxidative stress by the polyherbal may be the mechanism of action for such effects.

Keywords: Hepatotoxicity, antitubercular, Dawa-Ul-Kurkum, hydroalcoholic, liver

1. Introduction
The liver plays a crucial role in the metabolism and elimination of drugs and xenobiotics, and thus is very susceptible to the toxicity induced by them [1]. A wide range of drugs have been reported to induce liver dysfunction by various mechanisms and lead to hepatobiliary disorders [2]. The hepatotoxicity produced by anti-tubercular drugs is a common serious adverse drug reaction and is a challenging clinical problem [3, 4]. Such hepatic adverse effects of anti-tubercular therapy can result in poor compliance of patients to therapy which, in turn, may even lead to another important problem i.e., multi-drug resistant TB (where M. tuberculosis strain becomes resistant to two or more first-line drugs) [5].

Mycobacterium tuberculosis (TB)is one of the most dreaded diseases particularly with rapidly emerging multidrug-resistance that has assumed alarming dimensions in recent times [6]. About one-third of the world's population is infected with TB and nearly three million people per year are killed by this disease [7]. Anti-tubercular drug therapy (ATT)forms the cornerstone of treatment of this communicable disease [8]. Standard treatment for the tuberculosis (TB) consist of six months course of anti-TB drugs in the form of combination therapy which may vary depending upon various factors *viz*. the patient's age, type of TB infection, and whether they have been treated before. The 6-month long treatment consists of two phases: an initial intensive phase for 02 months, and a subsequent continuous phase of 04 months. In naïve patients, the initial 02 months of intensive phase starts with the first line agents like isoniazid, rifampicin, pyrazinamide, and ethambutol or streptomycin; followed by 04 months of continuous phase treatment with isoniazid and rifampicin only. Starting combination therapy with four drugs leads to destruction of strains of mycobacteria in all growth stages and then continuation therapy abolishes any residual dormant mycobacterial strain [5].

The most common adverse effect of anti-tubercular drug therapy is hepatotoxicity which sometimes may be so fatal that leads to hospitalization and life-threatening problems [3, 4]. Mostly the first line therapy drugs: isoniazid, rifampicin, and pyrazinamide are metabolized by the liver, and are potentially hepatotoxic [9] and, when used in combination (as in TB),chances of hepatotoxicity are increased manifold [1].

Hepatotoxicity caused by drugs may be mediated through alterations in oxidant-antioxidant balance which leads to oxidative stress and lipid peroxidation [10]. Several strategies have been explored for attenuation of anti-TB drug induced liver damage, but no satisfactory solution has been found for this important clinical problem.

Herbal drugs are emerging as strong alternatives or adjuncts to conventional modern medical therapy for many diseases. There has been renewed interest in the traditional remedies due to comparative lesser adverse effects combined with the regulatory issues arising out of the TRIPPS agreement. In recent years, complementary and alternative medicinal strategies using medicinal plants for prevention and treatment of diseases have been gaining importance. Further, application of modern medical technologies to traditional medicine has further authenticated/validated the use of these several mono- and polyherbal agents in a variety of complex pathophysiological states. A large number of medicinal plants have been used traditionally for immunomodulation and hepatoprotection and these effects need to be validated following modern scientific methodology. In Unani system of medicine, a polyherbal formulation Dawa-ul-Kurkum is effectively used in cases of liver dysfunction, anorexia, ascites and abdominal pain. This polyherbal is composed of Sunbul-ut-Teeb *(*Nardostachysjatamansi DC.), Mur Makki (CommiphoramyrrhaNees), Saleekha (Cinnamomum cassiaNees), Qust (Saussurealappa C.B. Clarke), Shagufa-e-Izkhir (Cymbopogonshoenanthus L.), Darcheeni (Cinnamomumzylenicum BL**.)**, Zafran (Crocus sativa L.) with Sharab-e-musallas (Ethyl alcohol) and QandSafaid (Saccharumofficinarum L.) [11, 12]. The present study has thus been designed to evaluate the possible hepatoprotective effects ofDawa-ul-Kurkum and its 50% hydroalcoholic extract in anti-TB drug induced hepatotoxicity in rats and to delineate the possible mechanisms involved in these effects.

2. Materials and methods

2.1 Animals

Inbred male Wistar rats (180-250 g), of either sex were used for the experiments. They were maintained in standard laboratory conditions *viz*. 12h light – 12h dark cycle (lights on at 8 AM) and had free access to food and water during the entire study duration. Care of animals was as per guidelines of CPCSEA (Govt. of India) for scientific research. The study protocol had the approval of the Institutional Animal Ethics Committee (IAEC) of the institute in accordance with Principals of Laboratory Animal care. The study approval number by IAEC is VPCI/IAEC/2017/13.

2.2 Drugs and Chemicals

2.2.1 The Investigational Drug

The standardized drug, Dawa-ul-Kurkum, was prepared and provided by Central Research Institute of Unani Medicine (CRIUM), Hyderabad, Ministry of AYUSH, Govt. of India with a batch no. 3-1/2018-19/CRIUM. This polyherbal is composed of Sunbul-ut-Teeb *(Nardostachysjatamansi* DC), Mur Makki (Commiphoramyrrha Nees), Saleekha (Cinnamomum tamala), Qust (Saussurealappa), Shagufa-e-Izkhir (Cymbopogon shoenanthus), Darcheeni (Cinnamomumzylenicum bark), Zafran (Crocus sativa) with Sharab-e-musallas (Saussureacostus**)** and QandSafaid (Saccharum offininarum) Q.S.

The formulation is well documented in standard Unani literature [13] and is certified to have been prepared as per traditional classical Unani text by CRIUM.

2.2.2 Other drugs and chemicals

The anti-TB drugs *viz*. rifampicin, isoniazid and pyrazinamide were obtained from Lupin Labs (Mumbai) and Silymarin was procured from Sigma-Aldrich (USA). The biochemical assay kits were purchased from ERBA Diagonostic, Mannheim, Gmbh, and other routine chemicals for experiments were procured from SRL, New Delhi.

2.3 Induction of Anti-tubercular drug induced hepatotoxicity

Hepatotoxicity was induced by daily oral administration of a combination of anti-TB drugs *viz*. Isoniazid (H, 30.85mg/kg), Rifampicin (R, 61.7mg/kg) and Pyrazinamide (Z, 132.65mg/kg), daily for 28 days [6]. The treatment groups were as follows: Group 1 (n=3, controls; vehicle, 2% gum acacia); Group 2 (n=3, experimental control; HRZ); Group 3 (n=5, Silymarin (50mg/kg) + HRZ; positive control); Group 4 & 5 (n=5 in each case, Dawa-ul-kurkum (DK) at dose 250 or 500 mg/kg respectively + HRZ); Group 6 & 7 (n=5, in each case, 50% hydroalcoholic extract of DK (HA-DK) at dose 500 or 1000 mg/kg + HRZ). The numbers of rats in each group were used as approved by the IAEC. The dose of Dawa-ul-Kurkum was calculated from the human dose as being traditionally used by the Unani physicians. All drugs were administered orally for 28 days. On 28th day, animals were anesthetized (ketamine/xylazine) and blood was collected by cardiac puncture. The blood was centrifuged for separating cellular components and stored at -80ºC for various immunological markers. After blood collection, animals were sacrificed and liver was excised out and stored for histopathological studies and estimation of biochemical and oxidative stress parameters. The caracas of the sacrificed animals were disposed of as per the guidelines of CPCSEA.

2.4 Biochemical estimations

Liver function test parameters, *viz*. serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP) were estimated by kinetic method by International Federation of Clinical Chemistry (IFCC). Serum bilirubin and total proteins were estimated by End Point assay as per the instruction of the Kit Manufacture's manual.

2.5 Assay for Malondialdehyde (MDA) levels

Malondialdehyde (MDA) is an establised biomarker for lipid peroxidation and oxidative stress. This was measured spectrophotometrically as 2-thiobarbituric acid-reactive substance (TBARS) in supernatant as per the method of Satoh *et al*. [14]. Briefly, 0.1 ml of supernatant samples was mixed with 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% 2-thiobarbituric acid. The reaction mixture was finally made up to 4.0 ml with distilled water. After vortexing, samples were incubated for 1 h in 95º C and after cooling with tap water; 1.0 ml of distilled water and 5.0 ml of mixture of butanol–pyridine 15:1 (v/v) was added. The mixture was shaken for 10 min. and then centrifuged at 4000 rpm for 10 min. Butanol–pyridine layer is measured spectrophotometrically at 532 nm. TBARS values are expressed as MDA equivalents. 1,1,3,3-tetramethoxypropane (TMP) was used as the standard.

2.6 Assay of reduced glutathione (GSH) levels

Reduced glutathione (GSH) levels were estimated by the method of Ellman [15]. This assay is based on the enzymatic recycling procedure in which glutathione was sequentially

oxidized by the DTNB and reduced by NADPH in the presence of glutathione reductase. Briefly, an equal quantity of sample was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.1 ml of this supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5'5-dithiobis (2-nitrobenzoic acid) and 0.4 ml of double distilled water was added. The mixture was vortexed and absorbance was read at 412 nm within 15 min. The concentration of 2-nitro-5-benzoic acid formation was measured and reduced glutathione is expressed as µmol/mg protein.

2.7 Assay for Nitrates and Nitrites (NOx)

Nitrates and nitrites (NOx) are stable metabolites of NO. NOx levels were determined by using the Griess reaction as described previously by Tracey *et al.* [16]. Briefly, 6µl of sample/supernatant was mixed with 44µl of distilled water, 20µl of 310 mM phosphate buffer (pH 7.5) and 10µl each of 0.86 mM NADPH, 0.11 mMflavin adenine dinucleotide and of nitrate reductase (FAD), 10µl Nitrate reductase (1 U/ml) in individual wells of a 96-well plate. Plate was thereafter incubated for 1 h at room temperature in the dark. 200µl of Griess reagent [1:1 mixture of 1% sulfanilamide (1% solution with 5% orthophosphoric acid) and 0.1% N(1-naphthyl) ethylenediamine (NEDA) (1% solution with distilled water)] was added to each well and the plate was incubated for an additional 10 min at room temperature. Absorbance was measured at 540 nm using a microplate reader. Total protein was estimated by method of Lowry *et al.* [17]. Concentration of total nitrate and nitrite (NOx) in liver homogenates was calculated from the standard curve and expressed as nM/mg protein.

2.8 Histopathological examination

Liver tissue samples of all the groups were subjected to histological examination. Microscopic examination was done by a qualified pathologist using hemotoxylin and eosin staining in a blinded fashion.

2.9 Statistical Analysis

All values were expressed as Mean ± SE. The data was analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey`s test for inter group comparisons. A p value of at least 0.05 was considered as level of significance in all statistical tests.

3. Results

3.1 Effects of Dawa-ul-Kurkum and its hydro-alcoholic extract on Liver Function tests (LFT) in anti-TB drug induced hepatotoxicity

In experimental control group, daily administration of anti-TB drugs, H+R+Z suspension (isoniazid 30.85mg/kg + rifampicin 61.7mg/kg + pyrazinamide 132.65mg/kg, p.o.) for 28 days resulted in significant increases (two-fold) in serum levels of SGOT (P<0.05), SGPT (p<0.01), total bilirubin(p<0.001), direct bilirubin (p<0.01) and reduction in total protein as compared to control rats. This was suggestive of notable degree of hepatotoxicity and tissue injury in the rat liver - thus validatingthis model of hepatotoxicity. In Groups 4 and 5,treatment with Dawa-ul-Kurkum (DK) at doses 250 and 500mg/kg, respectively, for 28 days, significantly attenuated the effects of anti-TB drugs and reduced level of serum SGOT, SGPT, ALP, total bilirubin and direct bilirubin (p< 0.05 in all cases) as compared to that in experimental control group (vehicle + anti-TB drugs). Similarly, in Group 6 and 7, treatment with 50% hydro-alcoholic extract of Dawa-ul-Kurkum (HA-DK, 500 and 1000mg/kg) produced hepatoprotective effects, as it significantly reduced the levels of serum SGOT, SGPT, ALP, total bilirubin and direct bilirubin (p<0.05, in all cases) as compared to that in experimental controls. However, nosignificant change was observed in the levels of total protein after the polyherbal agent.Pretreatment with silymarin also significantly reduced the hepatotoxic effects of anti-TB drugs and reduced the levels of serum SGOT, SGPT, ALP, total bilirubin and direct bilirubin (p<0.05, in all cases)as compared to that in experimental control. The results of DK and HA-DK were comparable to that of Silymarin. These results are summarized in Table1and Table 2.

Table 1: Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on SGOT, SGPT and ALP in anti-TB drug induced model of hepatotoxicity in rats

Treatment(mg/kg)	SGOT(IU/L)	SGPT (IU/L)	ALP(IU/L)
Control	105.5 ± 6.15	63.66 ± 3.76	102.7 ± 8.15
Experimental control	193.6 ± 8.93*	137.1 ± 12.14##	185.9 ± 60.67
Silymarin (50)	115.0 ± 13.80*	77.60 ± 8.62*	104.3 ± 7.89*
DK (250)	124.2 ± 13.19*	70.56 ± 11.64**	109.2 ± 5.61
DK (500)	113.9 ± 7.73*	79.43 ± 11.79*	103.6 ± 4.78*
HA (500)	122.5 ± 21.12*	79.77 ± 10.82*	111.8 ± 7.70
HA (1000)	128.7 ± 11.34	78.62 ± 10.23*	105.2 ± 6.88*

The values are expressed as mean ± SEM; DK-Dawa-ul-kurkum; HA-Hydroalcholic extract of DK. All groups except control group were treated with isoniazid 30.85mg/kg + rifampicin 61.7mg/kg + pyrazinamide 132.65mg/kg. ## p<0.01 when compared with control group; *p<0.05and **p<0.01 when compared with experimental control. The data were analyzed using one-way ANOVA followed by Tukey`s test.

Table 2: Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on total bilirubin, direct bilirubin and total protein in anti-TB drug induced model of hepatotoxicity in rats

Treatment mg/kg	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total protein (g/dl)
Control	0.34 ± 0.01	0.25 ± 0.017	7.28 ± 0.35
Experimental control	2.07 ± 0.50###	0.95 ± 0.02##	5.08 ± 0.32
Silymarin (50)	0.68 ± 0.17**	0.53 ± 0.07*	7.54 ± 0.46
DK (250)	0.74 ± 0.07**	0.46 ± 0.05**	8.63 ± 0.65*
DK (500)	0.95 ± 0.14*	0.55 ± 0.06*	7.36 ± 0.61
HA (500)	0.96 ± 0.13*	0.52 ± 0.05*	8.02 ± 0.83
HA (1000)	1.08 ± 0.23*	0.59 ± 0.10	7.52 ± 0.81

The values are expressed as mean ± SEM; DK-Dawa-ul-kurkum; HA-Hydroalcholic extract of DK. All groups except control group were treated with isoniazid 30.85mg/kg + rifampicin 61.7mg/kg + pyrazinamide 132.65mg/kg. ###p<0.001and ##p<0.01 vscontrol group; **p<0.01 and *p<0.05vs Experimental control. The data were analyzed using one-way ANOVA followed by Tukey`s test.

3.2 Effects of Dawa-ul-Kurkum and its hydro-alcoholic extract on body weight and liver weightin anti-TB drug induced hepatotoxicity

The mean body and liver weightswere recorded in all groups at 0 day and after 28 days of various drug treatments. The results showed that daily oral administration of anti-TB drugs (isoniazid 30.85mg/kg + rifampicin 61.7mg/kg + pyrazinamide 132.65mg/kg, p.o.) for 28 days caused significant reductions in the% change in body weight (p < 0.01), but nosignificant change in the liver weight when

compared to corresponding control rats. Interestingly, treatment with Dawa-ul-Kurkum (DK, 250 and 500mg/kg), 50% hydro-alcoholic extract of Dawa-ul-Kurkum (HA-DK, 500 and 1000mg/kg) and silymarinblocked the effects of anti-tubercular drugs and resulted in significant increase in the% change in body as compared to that in Experimental controls, the results are summarized in Table 3.

Table 3: Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on body weight and liver weight in anti-TB drug induced model of hepatotoxicity in rats

Treatment (mg/kg)	Initial body weight (g)	Final body weight (g)	% change in body weight	Liver weight (g) on 28th day	Liver index (%)
Control	247.7 ± 33.80	260.3 ± 32.87	4.84	8.21 ± 1.30	3.15
Experimental control	243.3 ± 16.91	219.7 ± 18.49	-10.74#	6.71 ± 0.42	3.05
Silymarin (50)	187.0 ± 12.14	207.0 ± 7.88	9.66*	6.89 ± 0.30	3.33
DK (250)	194.4 ± 17.70	211.2 ± 14.46	7.95*	7.29 ± 0.47	3.45
DK (500)	230.8 ± 11.63	236.6 ± 12.55	2.45*	7.28 ± 0.21	3.07
HA (500)	192.2 ± 19.65	201.4 ± 20.33	4.56*	7.08 ± 0.61	3.51
HA (1000)	188.0 ± 4.65	207.6 ± 9.64	9.44*	7.04 ± 0.58	3.39

The values are expressed as Mean ± SEM; DK-Dawa-ul-kurkum; HA-Hydroalcholic extract of DK. Initial and final body weight was measured on 0 and 28th day of treatment. All groups except control group were treated with isoniazid 30.85mg/kg + rifampicin 61.7mg/kg + pyrazinamide 132.65mg/kg. Liver index was calculated as (liver weight/body weight×100%); #p<0.01, when compared with control group; *P<0.01, when compared with Experimental control group. The data was analyzed using one way ANOVA followed by Tukey`s test.

3.3 Effects of Dawa-ul-Kurkum and its hydro-alcoholic extract on oxidative stress parameters in anti-TB drug induced hepatotoxicity

In experimental control group, daily administration of anti-TB drugsfor 28 days resulted in significant (P<0.05) increase in the levels of MDA, a marker for lipid peroxidationin theliver homogenate as compared to that in control rats. This was associated with an appreciable reduction in GSH levels. Further, elevations were also seen in levels of stable metabolites of nitric oxide (NOx) (P<0.05). In Group 4 and 5, treatment with Dawa-ul-Kurkum (DK) at doses 250 and 500mg/kg respectively for 28 days significantly attenuated the effects of anti-TB drugs and reduced level of MDA ($p < 0.05$ for both doses) as well as NOx ($p < 0.05$ for dose 500mg/kg), and significant increase in GSH ($p < 0.05$ for dose 500mg/kg), as compared to that in Experimental control group (treated with anti-TB drugs). Similarly, in Group 6 and 7 administration of 50% hydro-alcoholic extract of Dawa-ul-Kurkum (HA-DK, 500 and 1000mg/kg) attenuated the effects of anti-TB drugs and reduced the levels of MDAand NOx along with significant increase in GSH (p<0.05 for dose 1000mg/kg) as compared to that in Experimental control group. Pretreatment with silymarin also significantly reduced the levels of MDA, NOx, and increased GSH (p<0.05, in all cases) as compared to that in Experimental controls,which is suggestive of the hepatoprotective effects of the drug in this model. The results of Dawa-ul-Kurkum and its hydro-alcoholic extract were comparable to that of Silymarin. These results are summarized in Table 4.

Table 4: Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on oxidative stress parameters in anti-TB drug induced model of hepatotoxicity in rats

Treatment(mg/kg)	NOx (nmol/mg protein)	MDA (nmol/mgprotein)	GSH (µmol/mgprotein)
Control	0.15 ± 0.01	0.25 ± 0.03	2.43 ± 0.19
Experimental control	0.28 ± 0.05#	0.46 ± 0.03#	1.10 ± 0.02
Silymarin(50)	0.17 ± 0.17*	0.26 ± 0.02*	2.32 ± 0.16*
DK (250)	0.19 ± 0.02	0.28 ± 0.03*	2.16 ± 0.32
DK (500)	0.16 ± 0.01*	0.27 ± 0.01*	2.32 ± 0.25*
HA (500)	0.19 ± 0.03	0.35 ± 0.03	2.20 ± 0.17
HA (1000)	0.19 ± 0.01	0.33 ± 0.03	2.30 ± 0.27*

The values are expressed as mean ± SEM; DK-Dawa-ul-kurkum; HA-Hydroalcholic extract of DK; #p<0.05 vs control group; *p<0.05 vs Experimental control. All groups except control group were treated with isoniazid 30.85mg/kg + rifampicin 61.7mg/kg + pyrazinamide 132.65mg/kg. The data were analyzed using one-way ANOVA followed by Tukey`s test.

3.4 Effects of Dawa-ul-Kurkum and its hydro-alcoholic extract on histopathological changes in liver in anti-TB drug induced hepatotoxicity

Histopathologicalexamination of the liver sections of vehicle treated (control)ratsshowed fairly well-preserved lobular architecture in hepatic tissue with mildly congested central vein, hepatocyte and sinusoids. In experimental control group, daily administration of anti-TB drugs, H+R+Z suspension (isoniazid 30.85mg/kg + rifampicin 61.7mg/kg + pyrazinamide 132.65mg/kg, p.o.) for 28 days showed degeneration in the hepatocytes, fatty changes, focal areas of necrosis, mild vasodilation and perivascular infiltration of inflammatory cellson histological examination of rat livers.The normal radiating pattern of the hepatocytes was not preserved. Silymarintreated group showed fairly preserved hepatic parenchyma with focal area of dilated sinusoids having mild hemorrhages. Focal areas of hydropic degenerative changes were seen. In Group 4 and 5, treatment with Dawa-ul-Kurkum (DK) at doses 250 and 500mg/kg respectively for 28 days showed fairly preserved lobular architecture, radiating pattern of hepatocytes arrangement and very mild,insignificant, degenerative changes were seen. No

hemorrhages/inflammatory cell infiltrate and no sinusoid dilation were seen. It showed fairly well preserved lobular architecture and radiating pattern of hepatocytes arrangement was present. Hepatocytes mostly appeared normal and with no degenerative changes, although congestion of the central vein was seen in some parts.In Group 6 and 7 treatments with 50% hydro-alcoholic extract of Dawa-ul-Kurkum (HA-DK, 500 and 1000mg/kg) showed mild loss of lobular architecture. Radiating pattern of hepatocytes arrangement was not properly present and mild degenerative changes of the hepatocytes were evident. Congestion of the central vein was seen in some part. Focal area of hemorrhage was also evident. Mild inflammatory cells infiltrate was seen. The results are shown in Figure.1.

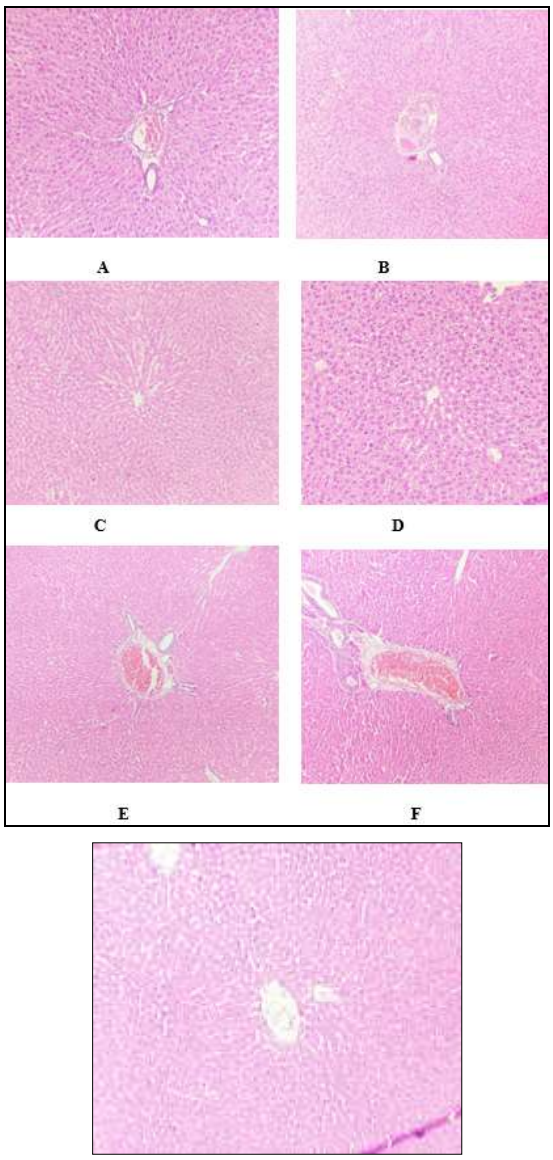

Fig 2: Histopathological picture of liver sections after various drug treatment in rats. (A) Control. (B) Experimental control. (C) Silymarin. (D) DK250. (E) DK500. (F) HA500. (G) HA1000. All groups except control group were treated with isoniazid 30.85 mg/kg + rifampicin 61.7 mg/kg + pyrazinamide 132.65 mg/kg. DK-Dawa-ul-kurkum; HA-Hydroalcholic extract of DK.

4. Discussion

Hepatotoxicity is a serious complication of anti-tubercular drugs (isoniazid, rifampicin, and pyrazinamide), and this is compounded several times when they are given in combination for a long period (as in TB).Liver damage produced by anti-TB drugs has been attributed to oxidative stress and free radical damage to hepatocytes [18]. Oxidative stress is produced due to disruption in the balance between anti-oxidant enzymes and reactive oxygen species, tilting the scales in favor of the latter. Free radicals are initiated due to interactions of reactive metabolites of isoniazid, rifampicin, and pyrazinamide (mono/diacetyl hydrazine, 3-formyl rifampicin, pyrazinoic acid) with oxygen or superoxide radicals and H_2O_2. They result inper-oxidative degradation of membrane lipids and endoplasmic reticulum, which are rich in poly-unsaturated lipids and fatty acids. This leads to creation of peroxides of lipids which in turn form substances such as MDA, which results inloss of integrity of cell membrane and destruction to liver tissues [19]. Nitric oxide (NO)is a ubiquitous free radical moiety, which was first discovered in the vascular endothelium and now known to be located in many tissue/organ systems including the gastrointestinal and hepatobiliary system. Increased levels of NO are found in inflammatory conditions [20]. In experimental control group of rats, liver damage was characterized by a rise in serum SGOT, SGPT, ALP, total bilirubin and direct bilirubin levels but decrease in serum protein level. Oxidative stress parameters like tissue MDA and NOxlevels were concurrently increased which was accompanied by lowered GSH levels. Rise in MDA level indicated increased lipid peroxidation which was accompanied with decrease in tissue GSH levels. This indicated failure of anti-oxidant defense system to scavenge excess free radicals. The decrease in level of proteins in anti-TB drug treated group may have beendue to liver dysfunction following decrease in levels of reduced glutathione in the tissues leading to inhibition of many enzymes containing SH group and inhibition of protein synthesis [19], which is an indicator of severe liver damage [21, 22]. The biochemical findings were corroborated by the histopathological examination of liver tissue which showed degeneration, necrosis, fibrosis and inflammatory changes in rat hepatic tissue.

The present results showed that concurrent administration of Dawa-ul-Kurkumand 50% Hydro-alcoholic extract of Dawa-ul-Kurkumalong with anti-tubercular drugs significantly prevented the rise in the level of serum SGOT, SGPT, ALP, total bilirubin and direct bilirubin. Further, measurement of oxidative stress parameters in liver homogenates showed protective effects of Unani polyherbal preparartion, Dawa-ul-Kurkum (DK)against raised levels of reactive oxygen and nitrogen species in response to anti-TB drugs – as seen by lowered levels of MDA and NOx and elevating the levels of GSH. The effects with the HA extract of DK were less consistent as compared to that seen with DK, and only some of the parameters tested were attenuated. Histopathological examination of liver also showed that DK preserved lobular architecture and radiating pattern of hepatocytes and they mostly appeared normal with no degenerative changes, thus reemphasizing the protective effect of this polyherbal formulation against the anti-TB drug induced hepatotoxicity. However, as seen in the biochemical studies, the protective effect were not that prominent withthe hydro-alcoholic extract of Dawa-ul-Kurkum (HA-DK) and its administration showed mild loss of lobular architecture, mild degenerative changes of the hepatocytes and mild inflammatory cells infiltrate in some parts. These results showed that Dawa-ul-Kurkum and to some extent it`s HA preparation was effective as hepatoprotective agent and prevented the anti-tubercular drugs induced hepatotoxicity. The protective effects may be mediated through restoring/maintenance of the anti-TB drug

induced disruption of the pro-oxidant/anti-oxidant homeostatic balance.

5. Conclusions

The present study demonstrated that the polyherbalUnani formulation, Dawa-ul-Kurkum (DK) was effective against theanti-TB drugs induced hepatotoxicityin rats as it significantly attenuated the biochemical, histopathological and oxidative stress markers of hepatic damage. However, such consistent effects were not seen with the hydro-alcoholic extractof DK. The study is of considerable translational value as adopting such reverse pharmacology approach could help in the integration of traditional and modern medicinal concepts in the greater interest of drug development and rationalizing therapy for hepatotoxicity especially in patients on anti-TB therapy.

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7. Abbreviation

ATT, Anti-tubercular drug therapy; TB, Tuberculosis; IAEC, Institutional Animal Ethics committee; H, Isoniazid; R, Rifampicin; Z, Pyrazinamide;DK, Dawa-ul-kurkum; HA, Hydroalcoholic extract;ALT, Serum alanine aminotransferase; AST, Serum aspartate aminotransferase; ALP, Serum alkaline phosphatase; IFCC, International Federation of Clinical Chemistry; MDA, Malondialdehyde; TBARS, 2-thiobarbituric acid-reactive substance; TMP, 1,1,3,3-tetramethoxypropane; GSH, Reduced glutathione; NOx, Nitrates and Nitrites; FAD, Flavin adenine dinucleotide; ANOVA, One-way analysis of variance; and LFT, Liver Function tests

8. Contribution of the authors

Mohd. Rafi Reshi was involved in the conduct of experiments, acquisition and analysis of data and drafting of the manuscript. Kavita Gulati was involved in conceptualization, planning and designing of the study. She also helped in the analysis of data and critical review of the manuscript. Asim Ali Khan was involved in the critical reviewing of manuscript.ArunabhaRay was involved in planning of the study, interpretation of data and critical reviewing of manuscript. All authors approved the final version of the manuscript.

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